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March 8, 2004

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America for the term set forth below, subject to the payment of maintenance fees as provided by law.

If this application was filed prior to June 8, 1995, the term of this patent is the longer of seventeen years from the date of grant of this patent or twenty years from the earliest effective U.S. filing date of the application, subject to any statutory extension.

If this application was filed on or after June 8, 1995, the term of this patent is twenty years from the U.S. filing date, subject to any statutory extension. If the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121 or 365(c), the term of the patent is twenty years from the date on which the earliest application was filed, subject to any statutory extension.

Bruce Lehman

Commissioner of Patents and Trademarks

Sandra L Morton

Attest

US005824796A

United States Patent [19]

Petrie et al.

[11] Patent Number: 5,824,796

[45] Date of Patent: Oct. 20, 1998

[54] **CROSS-LINKING OLIGONUCLEOTIDES**

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[21] Appl. No.: **334,490**

[22] Filed: **Nov. 4, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 49,807, Apr. 20, 1993, abandoned, which is a continuation of Ser. No. 353,857, May 18, 1989, abandoned, which is a continuation-in-part of Ser. No. 250,474, Sep. 28, 1988, abandoned.

[51] **Int. Cl.**[6] **C07H 19/04**; C07H 21/00; C07H 21/02; C07H 21/04

[52] **U.S. Cl.** **536/26.7**; 536/24.5

[58] **Field of Search** 536/26.1, 26.12, 536/26.13, 26.14, 26.8, 27.6, 27.81, 28.5, 28.54, 26.7, 24.5

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[57] **ABSTRACT**

This invention is directed to novel substituted nucleotide bases with a crosslinking arm which accomplish crosslinking between specific sites on adjoining strands of oligonucleotides or oligodeoxynucleotides. The invention is also directed to oligonucleotides comprising at least one of these crosslinking agents and to the use of the resulting novel oligonucleotides for diagnostic and therapeutic purposes. The crosslinking agents of the invention are of the following formula (I'):

$$R_1—B—(CH_2)_q—(Y)_r—(CH_2)_m—A' \quad (I')$$

wherein,

R_1 is hydrogen, or a sugar moiety or analog thereof optionally substituted at its 3' or its 5' position with a phosphorus derivative attached to the sugar moiety by an oxygen and including groups Q_1 Q_2 and Q_3or with a reactive precursor thereof suitable for nucleotide bond formation;

Q_1 is hydroxy, phosphate or diphosphate;

Q_2 is =O or =S;

Q_3 is CH_2—R', S—R', O—R', or N—R'R";

each of R' and R" is independently hydrogen or C_{1-6} alkyl;

B is a nucleic acid base or analog thereof that is a component of an oligonucleotide;

Y is a functional linking group;

each of m and q is independently 0 to 8, inclusive;

r is 0 or 1; and

A' is a leaving group.

This invention is also directed to novel 3,4-disubstituted and 3,4,-trisubstituted pyrazolo[3,4-d]-pyrimidines and to the use of these nucleic acid bases in the preparation of oligonucleotides. The invention includes nucleosides and mono- and oligonucleotides comprising at least one of these pyrazolopyrimidines, and to the use of the resulting novel oligonucleotides for diagnostic purposes.

15 Claims, 3 Drawing Sheets